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Impact of Heat Stress on the Fecal Shedding Patterns of *Salmonella enterica* Typhimurium DT104 and *Salmonella enterica* Infantis by 5-Week-Old Male Broilers

JOSIE L. TRAUB-DARGATZ,[1] SCOTT R. LADELY,[2] DAVID A. DARGATZ,[3] and PAULA J. FEDORKA-CRAY[2]

ABSTRACT

The objective of this study was to determine if there is an impact of heat stress of broiler chickens on number and survival of two types of *Salmonella* shed in the chicken's feces after an oral challenge. The data from this study indicate that heat stress did not result in higher levels or longer survival of S*almonella* spp. shed in feces. It is possible that the duration or intensity of the heat stress employed was not sufficient or that heat stress does not alter the number or survivability for these particular strains of *Salmonella* spp. Feces stored at room temperature after collection, resulted in the numbers of both strains of *Salmonella* increasing by one to three logs in the first week. This finding indicates that there could be an increase in environmental contamination under certain conditions.

INTRODUCTION

A PORTION OF BROILER CARCASSES are known to be contaminated with fecal pathogens such as *Salmonella* spp. when evaluated post-slaughter (USDA, 2005). It is plausible that heat stress affects not only the bacterial load in the feces of birds but also the duration and level of contamination in the environment where feces are passed since foodborne related outbreaks associated with *Salmonella* increase in summer months (Kessel et al., 2001; Lee and Middleton, 2003). If bacteria shed by heat-stressed animals were in higher numbers or more environmentally adapted, then intervention strategies to avoid heat stressors could be employed to lower the risk of outbreaks of salmonellosis in warmer months. Broiler production occurs throughout the year with 6–8-week production cycles (Simmons et al., 2003). Heat stress is a real possibility since the majority of the broiler production occurs in the Southeastern United States, where summer temperatures and humidity exceed 90°F and 90%, respectively.

Well-described experimental heat stress protocols for poultry (Bobek et al., 1997; Bollengier-Lee et al., 1999; Farnell et al., 2001; Lin et al., 2000) have been demonstrated to alter hormone levels and susceptibility to colonization with pathogenic bacteria (Bobek et al., 1997; Bollengier-Lee et al., 1999; Farnell et al., 2001; Lin et al., 2000). Various conditions have been evaluated to determine how bacteria respond to various stressors (such as pH and water availability), and survival in the environment may differ by type of bacteria (Leyer et

[1]Department of Clinical Sciences, Colorado State University College of Veterinary Medicine and Biomedical Sciences, Fort Collins, Colorado.
[2]Russell Research Center, Agricultural Research Station, U.S. Department of Agriculture, Bacterial Epidemiology and Antimicrobial Resistance Research Unit, Athens, Georgia.
[3]U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Centers for Epidemiology and Animal Health, Fort Collins, Colorado.

al., 1995; Mattick et al., 2000; Bell and Kyriakides, 2002; Gray and Fedorka-Cray, 2001; Guan and Holly, 2003; Plym and Ekesbo, 1996; Radkowski, 2002). However, reports regarding survival characteristics of fecal bacteria shed by experimentally challenged heat-stressed birds compared to birds kept under thermoneutral temperature conditions are lacking.

We undertook this pilot study in which 5-week-old male broilers were orally challenged with two different serotypes of *Salmonella* spp. to determine if there was a detectable difference in survival characteristics of bacteria in feces recovered from birds that were housed in ambient temperature versus those that were repeatedly heat stressed.

METHODS

Bacterial strains

A wild-type *Salmonella enterica* serotype Infantis that was associated with a previous large outbreak of disease among hospitalized large-animal patients and a wild-type *Salmonella enterica* serotype Typhimurium DT104 poultry isolate were used as challenge strains. At 5 weeks of age (day 0), broilers were challenged with 0.1 mL of *S*. Infantis (5×10^9) or *S*. Typhimurium DT104 (9×10^9), by oral gavage.

Animals

A total of 120 healthy 4-week-old male broilers was transported 15 min from a commercial source to the research facility in crates each containing 12 birds. Composite fecal samples were collected from the pens on the day after placement and cultured for *Salmonella* spp.

Experimental design

Four rooms were divided into two adjoining pens using solid plastic panels (each pen was 11 ft long by 54 in wide). Fifteen birds were placed per pen for a total of 30 birds per room. Biocontainment procedures were employed between each room to prevent any cross contamination between the groups. Birds in two rooms received either *S*. Infantis or *S*. Typhimurium DT104, with birds with one strain in one room (two pens) kept at ambient temperature and birds in the other room (two pens) subjected to repeated heat stress.

Birds had *ad libitum* access to feed (commercial scratch with no antimicrobials) and water throughout the study except for the immediate post-challenge period when feed was withheld for 12 h. The rooms with the birds in the ambient temperature/thermoneutral group were kept at temperature between 74.3°F and 76.0°F, while those housing the heat-stressed group had an 8-h period on days 0 and 2–7 when the temperature in the room averaged between 83.9°F and 88.7°F. The supplemental heat was provided by space heaters during the heat-stress period. The birds were routinely monitored during the heat-stress period to be sure the birds did not become excessively stressed. Relative humidity averaged approximately 32%. Birds were exposed to room light 24 h per day.

Statistical analysis

For analysis purposes the unit of observation was the pen. For each treatment (organism and temperature treatment) there were two pens. Given the limited number of observations per treatment, non-parametric statistics were used. The organism counts were converted to ranks (1–8) for each day of sampling. The ranks were then used in analysis of variance models that included independent variables for organism and environmental temperature. The organism counts for each evaluation (day of sampling and re-culture after storage) were analyzed in the same way. Since there is no described non-parametric equivalent of a repeated measures analysis, each analysis was conducted independently. In addition, the temporal change in estimated number of organisms in the stored samples was evaluated. The change in the estimated counts from the day of collection to the estimated counts after one week of storage were evaluated using the sign test.

The experiment was meant to be exploratory in nature and there were a limited number of observations per treatment group. As such, under most conditions, we would suggest a p value for significance to be at the 0.10 level. However, given the increased number of analy-

ses being conducted ($n = 16$), we elected to error on the side of conservatism and selected a p value for significance of 0.05. All analyses were conducted using SAS (version 9.1; SAS Institute, Cary, NC).

Pooled fecal sample collection, processing, and storage

Birds were housed on white freezer paper sheets covering the entire pen floor. Fresh paper was added in sequential layers the morning of each fecal collection day. Then feces were collected 6 h after placement of fresh paper on days 1, 3, 5, and 7 post-challenge as a single composite using a sterile wooden tongue depressor. Feces for each pen were combined into a sterile plastic whirl pack bag and vigorously mixed using external manual manipulation similar to stomaching, transferred into sterile 500-mL plastic bottles, covered with filter paper, and stored at room temperature in a biosecure area in the research laboratory.

Method of culture for baseline detection of Salmonella *in pooled sample*

One-gram aliquots of each fecal pooled sample were inoculated into GN Hajna broth (9 mL), which were incubated for 18–24 h at 37°C. Then 100 μL of GN Hajna broth was transferred to Rappaport-Vassiliadis medium, incubated for 18–24 h at 37°C, and streaked on XLT-4 agar plates. All XLT-4 agar plates were incubated for 24 h at 37°C, at which time colonies with typical appearance of *Salmonella* were inoculated into triple-sugar-iron and lysine iron agar slants for biochemical confirmation.

Salmonella *quantification method for pooled fecal specimens*

Quantitative bacteriology was conducted for the pooled fecal samples by using the five-tube most probable number method as previously described (Gray and Fedorka-Cray, 2001), and reported data are the mean colony-forming units (CFUs) by treatment group for each fecal collection day (days 1, 3, 5, and 7 post-challenge). Representative isolates from each group were sent to the National Veterinary Services Laboratories (NVSL; Ames, IA) for serotyping.

At the termination of the study on day 7 post-challenge, all birds were terminated using cervical dislocation. Cloacal swabs were collected from each bird to determine the prevalence of birds shedding *Salmonella* spp. Cloacal swabs were cultured for quantitative analysis utilizing the same enrichment broths, incubation scheme, and plating media described above without serial dilutions.

Storage and culture of collected feces

Feces stored in bottles covered with filter paper but open to the air from each of the collection days were quantitatively cultured for *Salmonella* spp. weekly for 3 weeks post-collection as described above.

RESULTS

Observations of birds' response to Salmonella *spp. challenge and heat stress*

Clinical signs were absent in all birds with the exception of during the heat-stress periods, when a few fecal pats intermittently had flecks of blood present. As birds were not housed separately, it was not possible to attribute the blood in feces to a specific bird; however, birds in the *S*. Typhimurium DT104 group, regardless of room temperature, had blood in their feces for 4 days, as compared to the *S*. Infantis group, which had blood in their feces on only 1 day.

Birds that were heat stressed exhibited less movement around the pen and had reduced feed intake during the heat-stress period than the birds in the thermoneutral temperature groups. The birds often would rest on their sternum in a group and were intermittently panting. All birds were able to rise if stimulated and none appeared extremely distressed or moribund. One bird in the *S*. Infantis heat-stress group was found dead at the end of the heat-stress period on day 7 of the study. The cause of death was not determined.

Culture results

Baseline pooled fecal cultures from pre-challenge samples. No *Salmonella* was detected in the baseline pooled fecal samples from any of the pens.

Individual bird cloacal sac contents. At the termination of the study on day 7 post-challenge, a greater proportion of the birds challenged with *S*. Typhimurium DT104 were still shedding (30/30 heat-stressed and 27/30 thermoneutral birds) as compared to those challenged with *S*. Infantis (21/29 heat-stressed and 18/30 thermoneutral birds).

Level of shedding of Salmonella *spp in fresh feces on days 1, 3, 5, and 7 post-challenge.* On day 1 post-challenge, the level of bacterial shedding (CFU/g) of *Salmonella* spp. was similar across the groups (Table 1), while an approximately 1-log difference between groups was observed on day 3 post-challenge (Table 1). On day 3, the birds challenged with *S*. Infantis and heat-stressed shed 1 log more than the ambient temperature group, while the birds challenged with *S*. Typhimurium and heat-stressed shed 1 log less than the ambient temperature group. On days 5 and 7, the number of *Salmonella* spp. (CFU/g) present in fresh pooled feces between the *S*. Infantis heat-stressed and ambient temperature groups differed by 2 logs, with the ambient temperature group shedding larger numbers. On days 5 and 7, *S*. Typhimurium DT104 heat-stressed and ambient temperature groups shed similar numbers of bacteria, which were higher than birds challenged with *S*. Infantis.

Survival of bacteria in stored feces

One week after collection and storage at room temperature as described above, the number of *Salmonella* spp. increased 1–3 logs compared to original collection day (Table 2). Replication in feces stored at room temperature in the laboratory was not anticipated 1 week post-storage; thus, the number of dilutions prepared only allowed for reporting of up to 4.7×10^4 CFU/g of feces in the week 1 post-collection samples for the day 1 and 3 post-challenge samples and up to 4.7×10^5 in the week 1 post-collection days 5 and 7 post-challenge samples.

By week 2 after collection and storage at room temperature as described above, the number of *Salmonella* spp. began to decline but was detectable in 10 out of 16 samples and ranged from 0.75×10^1 to 1.8×10^5 CFU/g. The highest survival numbers were observed in the *S*. Typhimurium DT104 ambient temperature day 3 post-challenge sample. By week 3 post-storage, *Salmonella* spp. were detected in only one sample at low levels of 1×10^1 CFU/g in the *S*. Typhimurium DT 104 day 3 post-challenge sample. The *Salmonella* types identified at NVSL were those inoculated into the birds at the initiation of the study.

Neither the organism nor the environmental treatment had a significant effect on the estimated number of organisms in the samples except for the samples collected on day 3 and held for 2 weeks. For this set of samples, those from the chickens that were kept at ambient temperature had significantly more ($p < 0.05$) organisms, regardless of the serotype inoculated, than did samples from chickens that were heat stressed.

Table 1. Most Probable Number for CFU/g of Fresh Feces on Days 1, 3, 5, and 7 Post-Challenge by Treatment Group

	Day			
Treatment group	*1*	*3*	*5*	*7*
S. Infantis, heat stressed	1.8×10^3	1.7×10^3	0.75×10^1	No growth
S. Infantis, ambient temperature	8.3×10^3	2.1×10^2	5×10^3	7.2×10^2
S. Typhimurium DT 104, heat stressed	4.9×10^3	1.3×10^3	2.3×10^5	2.8×10^3
S. Typhimurium DT 104, ambient temperature	7.9×10^3	1.1×10^4	7.5×10^4	4.6×10^3

CFU/g, colony-forming units per gram.

Table 2. Most Probable Numbers for CFU/g for Fecal Samples Cultured at 1, 2, and 3 Weeks After Collection

Treatment group	*Day 1 post-challenge*	*Day 3 post-challenge*	*Day 5 post-challenge*	*Day 7 post-challenge*
S. Infantis, heat stress, 1 week post-collection	$>4.7 \times 10^4$	$>4.7 \times 10^4$	6.1×10^3	2.1×10^3
S. Infantis, ambient temperature, 1 week post-collection	$>4.7 \times 10^4$	$>4.7 \times 10^4$	$>4.7 \times 10^5$	$>4.7 \times 10^5$
S. Typhimurium, heat stress, 1 week post-collection	$>4.7 \times 10^4$	$>4.7 \times 10^4$	$>4.7 \times 10^5$	3.7×10^5
S. Typhimurium, ambient temperature, 1 week post-collection	$>4.7 \times 10^4$	$>4.7 \times 10^4$	$>4.7 \times 10^5$	2.6×10^5
S. Infantis, heat stress, 2 weeks post-collection	No growth	3.3×10^3	No growth	No growth
S. Infantis, ambient temperature, 2 weeks post-collection	7×10^3	6.1×10^2	1.3×10^1	No growth
S. Typhimurium, heat stress, 2 weeks post-collection	3.3×10^1	3.4×10^3	2×10^2	No growth
S. Typhimurium, ambient temperature, 2 weeks post-collection	1.9×10^3	1.8×10^5	0.75×10^1	No growth
S. Typhimurium DT 104, ambient temperature, 3 weeks post-collection[a]	No growth	1×10^1	No growth	No growth

[a]All other week 3 post-collection samples were negative for *Salmonella* spp.
CFU/g, colony-forming units per gram.

The change in the estimated number of organisms in the fecal samples stored for 1 week were significantly higher ($p < 0.05$) for samples collected at each time period post-inoculation (e.g., day 1, day 3, day 5, and day 7).

DISCUSSION

The goals of this study were to determine if heat stress of broilers resulted in higher levels of shedding of *Salmonella* spp. in feces and if the *Salmonella* spp. shed by heat-stressed birds survived longer when compared to *Salmonella* spp. shed by birds kept at a typical ambient temperature. Results from this study indicate that heat stress did not result in a consistent trend for a larger number of *Salmonella* spp. in the pooled feces of birds challenged with *S.* Infantis or *S.* Typhimurium DT104, nor did heat stress of the birds result in longer persistence of the *Salmonella* spp. in feces collected from birds and stored.

It is possible that the duration or intensity of the heat stress employed in this study was not sufficient to result in changes in the salmonellae shed or that heat stress does not alter the number or survivability for these particular strains of *Salmonella* spp. shed in feces.

Conducting a study using longer periods of heat stress per day or achieving a higher heat index, or employing quantification of *Salmonella* spp. in individual bird samples could result in detection of a difference between number and survivability of these particular strains of *Salmonella* spp. shed in feces of heat-stressed birds compared to those kept at ambient temperatures.

One interesting finding in this study was the marked increase in number of *Salmonella* spp. (CFU/g) present in feces stored for 1 week under ambient room temperature and humidity conditions of the laboratory as compared to the number present in freshly passed feces. This finding implies that the number of pathogenic bacteria can increase under environmental conditions similar to those in an animal housing situation. Further studies to determine conditions that alter the number of *Salmonella* spp. in fecal material (CFU/g) under conditions that would mimic those in animal housing areas are warranted.

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Address reprint requests to:
Josie L. Traub-Dargatz, D.V.M., M.S.
Department of Clinical Sciences
Colorado State University College of Veterinary Medicine and Biomedical Sciences
Fort Collins, CO 80523

E-mail: jtraub1@lamar.colostate.edu